

CLAIMS

We claim:

1. A method of assaying target oligonucleotides in a sample comprising the steps of:

(a) contacting at least one capture oligonucleotide with the sample under suitable hybridization conditions to hybridize the target oligonucleotide to the capture oligonucleotide, the capture oligonucleotide comprising a molecular recognition sequence comprising at least one non-standard base, the capture oligonucleotide being coupled to a support, the target oligonucleotide comprising a tagging sequence complementary to the molecular recognition sequence of the capture oligonucleotide and an analyte-specific sequence or a complement of the analyte-specific sequence; and

(b) detecting hybridization of the target oligonucleotide to the capture oligonucleotide.

2. The method of claim 1, wherein the support comprises a single solid support.

3. The method of claim 1, wherein the support comprises a solid particle.

4. The method of claim 1, wherein the support comprises at least two different types of capture oligonucleotides, the sequences of each type of capture oligonucleotide differing from the molecular recognition sequence of other types of capture oligonucleotides by at least one base, and wherein the tagging sequence of the target oligonucleotide is complementary to the molecular recognition sequence of one of the types of capture oligonucleotides.

5. The method of claim 1, wherein at least two different types of capture oligonucleotides are coupled to a plurality of supports, the molecular recognition sequences of each different type of capture oligonucleotide differing by at least one base and wherein each target oligonucleotide comprises a tagging sequence complementary to the molecular recognition sequence of one of the types of capture oligonucleotides.

6. The method of claim 5, wherein the plurality of supports form at least two groups, each group having characteristic that distinguishes its members from members of others groups.

7. The method of claim 6, wherein the capture oligonucleotides on each group of supports have a different sequence than those on other groups of supports.

8. The method of claim 1, wherein the target oligonucleotide further comprises a reporter or coupling moiety and wherein detecting step comprises detecting the presence of the reporter or coupling moiety associated with the support.

9. The method of claim 8, wherein the reporter or coupling moiety is associated with a non-standard base.

10. The method of claim 1, wherein the contacting step is conducted under conditions of moderate or higher stringency.

11. The method of claim 10, wherein wherein the contacting step is conducted under high stringency conditions.

12. The method of claim 1, wherein at least one target oligonucleotide is prepared by

contacting an analyte with at least one primer pair, the analyte comprising at least one analyte-specific sequence, each primer pair comprising a first primer and a second primer, the first primer comprising a target oligonucleotide tagging sequence and a sequence complementary to a first sequence of the analyte, the second primer comprising a sequence complementary to a second sequence of the analyte; and

enzymatically extending the first and second primers to form the target oligonucleotide and a second oligonucleotide, wherein one of the target oligonucleotide and the second oligonucleotide comprises the analyte-specific sequence and the other comprises a sequence complementary to the analyte-specific sequence.

13. The method of claim 12, wherein the second oligonucleotide comprises a reporter or coupling moiety .

14. The method of claim 1, wherein the tagging sequence of the target oligonucleotide comprises a first sequence and a second sequence, the first sequence located at the 5' end of the target oligonucleotide and the second sequence located at the 3' end of the target oligonucleotide.

15. The method of claim 12, wherein the at least one of the first and second primers is a first allele-specific primer, further comprising contacting the analyte with at least one additional allele-specific primer different from the first allele-specific primer.

16. The method of claim 12, wherein both the target oligonucleotide and the second oligonucleotide comprise a tagging sequence and the capture oligonucleotide comprises a first molecular recognition sequence and a second molecular recognition sequence coupled to the first molecular recognition sequence through a linker, the first and second molecular recognition sequences being complementary to the tagging sequences of the target oligonucleotide and the second oligonucleotide, respectively.

17. The method of claim 12, further comprising covalently coupling the second oligonucleotide to the capture oligonucleotide and removing the target oligonucleotide.

18. The method of claim 17, wherein the step of covalently coupling comprises ligating the second oligonucleotide to the capture oligonucleotide.

19. A method of assaying a target oligonucleotide in a sample comprising steps of:

(a) contacting a capture oligonucleotide coupled to a support with the sample under suitable hybridization conditions to hybridize a target oligonucleotide, the target oligonucleotide comprising a tagging sequence comprising at least one non-standard base and an analyte-specific sequence or a complement thereof, the capture oligonucleotide

comprising a molecular recognition sequence comprising a sequence that is the same as or complementary to the analyte-specific sequence;

(b) enzymatically extending the capture oligonucleotide using the target oligonucleotide as a template and incorporating a complementary non-standard base opposite the non-standard base of the tagging sequence; and

(c) incorporating a reporter or coupling moiety onto an extended portion of the capture oligonucleotide, and

(d) detecting the presence of the target oligonucleotide in the sample by detecting incorporation of the reporter or coupling moiety.

20. A method of assaying a target oligonucleotide comprising steps of:

(a) contacting an analyte comprising the analyte-specific sequence with a first primer and a second primer, the first primer comprising a tagging sequence and a sequence complementary to a first sequence of the analyte, the second primer comprising a sequence complementary to a second sequence of the analyte and a non-standard base;

(b) enzymatically extending the first and second primers to form a target oligonucleotide and a second oligonucleotide, wherein one of the target oligonucleotide and the second oligonucleotide comprises the analyte-specific sequence and the other comprises a sequence complementary to the analyte-specific sequence, wherein the extension of the first primer is substantially halted at the position opposite the non-standard base of the second primer;

(c) incorporating a complementary non-standard base into the extended first primer opposite the non-standard base of the second primer;

(d) contacting a capture oligonucleotide coupled to a support with the target oligonucleotide under hybridizing conditions to hybridize a target oligonucleotide, the target oligonucleotide comprising the tagging sequence and the analyte-specific sequence or a complement of the analyte-specific sequence, the capture oligonucleotide comprising a molecular recognition sequence that is the same as or complementary to at least a portion of the analyte-specific sequence; and

(e) detecting hybridization of target oligonucleotide to the capture oligonucleotide.

21. A method of simultaneously detecting at least two alleles in a sample comprising genomic DNA comprising the steps of:

- (a) contacting the sample with at least two primer pairs comprising a first primer and a second primer under conditions such that each first and second primer of the primer pairs hybridizes to the genomic DNA;
- (b) amplifying the DNA sequences flanked by the first and second primer of each primer pair;
- (c) hybridizing the amplified DNA sequences of step (b) with at least two tagged, allele-specific primers, each tagged, allele specific primer comprising, in 5' to 3' order, a 5' tagging sequence comprising at least one non-standard nucleotide, a linker, and a 3' sequence capable of hybridizing with an amplified sequence of step (b);
- (d) enzymatically extending the allele-specific primer of step (c) in the presence of a labeled triphosphate base to form labeled extension products;
- (e) contacting the extension products of step (d) with at least two capture oligonucleotides coupled to a support under suitable hybridization conditions to hybridize an extension product, the extension product comprising a tagging sequence comprising at least one non-standard base and an allele-specific sequence, the capture oligonucleotide comprising a molecular recognition sequence comprising a sequence complementary to the tagging sequence, the molecular recognition sequence comprising a non-standard base complementary to the non-standard base of the; and
- (f) detecting the hybridization of at least one extension product to at least two capture oligonucleotides.

22. The method of claim 21, wherein one primer of each primer pair of step (a) comprises a first non-standard base, wherein the extension product of the primer is complementary to the allele-specific primer of step (c), wherein a labeled non-standard triphosphate base of step (d) is complementary to the first non-standard base of the primer of step (a), and wherein the extension product of step (d) comprises a labeled second non-standard base opposite the first non-standard base of the extension product of the primer comprising the first non-standard base.

23. The method of claim 21, wherein one primer of each primer pair of step (a) comprises, in 3' to 5' order, a 3' region that hybridizes with the genomic DNA of step

(a), a non-standard base that does not hybridize to the genomic DNA, and a 5' sequence non-complementary to the genomic DNA, wherein the extension product of the primer is complementary to the allele-specific primer of step (c), wherein the extension of product of step (d) does not include a complementary non-standard base opposite the non-standard base of the primer, region that first non-standard base

24. A method of assaying a target oligonucleotide comprising steps of:

(a) contacting an analyte comprising the analyte-specific sequence with a first primer and a second primer, the second primer comprising, in 5' to 3' order, a non-complementary sequence, a non-standard base, and an analyte-specific sequence;

(b) enzymatically extending the primers to form a target oligonucleotide and a second oligonucleotide, wherein one of the target oligonucleotide and the second oligonucleotide comprises the analyte-specific sequence and the other comprises a sequence complementary to the analyte-specific sequence;

(c) hybridizing to the extension product of the second primer of step (b) a tagged, allele-specific primer comprising, in 5' to 3' order, a 5' tagging sequence comprising a non-standard base, a linker, and a 3' sequence complementary to the extension product of the second primer;

(d) enzymatically extending the allele-specific primer of step (c);

(e) hybridizing a reporter oligonucleotide complementary to the 5' non-complementary sequence of the second primer of step (a) to the extension product of step (d), the reporter oligonucleotide comprising a 5' phosphate and a reporter moiety, to form a nick structure suitable for ligation by a ligase;

(f) contacting the nick structure of step (e) with a ligase to ligate the reporter oligonucleotide to the allele-specific extension product;

(g) contacting a capture oligonucleotide coupled to a support with the ligation product of step (f); and

(h) detecting hybridization of ligated oligonucleotide to the capture oligonucleotide

25. A kit for assaying an analyte, the kit comprising:

a support,

capture oligonucleotides coupled to the support, the capture oligonucleotides comprising a molecular recognition sequence having at least one non-standard base;

first primers comprising a tagging sequence and a sequence complementary to a first sequence of the analyte; and

second primers comprising a sequence complementary to a second sequence of the analyte.